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The emergence of peptides in the pharmaceutical business: From exploration to exploitation

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ABSTRACT

This minireview touches upon the challenges and opportunities peptides experience on the track to become an approved pharmaceutical.

Peptide attributes originally considered troublesome with respect to drug development may now turn out to be more convenient rather than unfavourable.

Besides characteristic high target affinity, biological peptides often exhibit higher than expected stability. Clearly natural selective pressure has optimised these biomolecules beyond what can be anticipated solely on the basis of their chemical nature. This concept is gradually finding its way into the pharma and biotech industry, as illustrated by a rise in medicinal peptide patent applications and developmental work.

1. Introduction

Drug development pipelines, which in the first century of the industry have been dominated by small molecules, are characterised by high attrition rates. The road to market authorisation has many obstacles and next to efficacy and tolerability, new drug candidates have to meet several other requirements. Besides essential pharmacodynamics, pharmacokinetics, toxicity, and safety issues, also economic factors are vital, including producibility, market competition, intellectual property, and others. This is why in a typical drug development process of today, >90% novel drug candidates fail between their identification and being put on the market.

As peptides are readily degraded inside the human body, which is equipped with roughly 600 molecularly different proteases [37], this class of (bio)chemicals has long been held ineligible for drug development, and deemed widely inferior to small molecules. Despite such neglect, a number of recent technological breakthroughs and advances have sparked major interest in their usage both as diagnostics as well as therapeutics. In particular, modern-day analytical methods, which greatly excel in sensitivity, resolution and throughput over those available to the traditional pharmaceutical

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industry, facilitate the discovery and identification of a wealth of novel peptides with pharmaceutical potential. Furthermore, present combinatorial chemistry provides the means to modify them and to create completely artificial variants and alternatives. Some pharmacodynamic 'weaknesses' of peptides cannot be fully abolished this way, but clever formulations may mask or amend them. In combination with an in-depth study of the complete biology of peptides in their original natural sources, current (bio)technologies have the potential to generate an ample spectrum of efficacious and safe peptide drugs.

Here, we review the steady rise of therapeutic peptides which is apparent in the pharmaceutical and biotech industry of today. We will focus on how a peptide drug candidate passes the various phases in the traditional pharmaceutical development pipeline and the differences herein to both small molecules and the larger biopharmaceuticals. With this, we aim to reveal that peptides are by far not 'undrugable', but, instead, offer immense medicinal potential.

2. Concept/history

In terms of chemical complexity, peptides fill a niche between typical small molecule chemicals and the larger proteins. Just as the latter, they feature a modular structure with amino acids linked by peptide bonds as base units. Their size is limited, with arbitrary boundaries set at up to 100 residues [20]. Nonetheless, within these limits, peptides exhibit multifarious structures with regard to amino acid sequence, post-translational modifications and resultant spatial shape.

Starting about a century ago (World War I), the advent of the modern drug era came with pioneering therapeutic compounds like the opiate morphine and the cyclic peptide penicillin, followed in the early 1920s by the (poly)peptide insulin. These drugs introduced a new standard in disease treatment. Although peptides thus held their place among the initial therapeutic discoveries [50], small molecules rapidly took preference in the drug development industry, primarily due to their ease of production, simplicity of administration (as oral 'pill') and superior pharmacodynamic properties. Meanwhile, the rapid enzymatic breakdown of peptides in biological systems and the consequently more challenging administration routes (e.g. injection such as for insulin) led to more and more neglect of this biochemical class in the traditional drug development process.

In the 21st century, the pharmaceutical business is experiencing dramatic changes. Stringent safety regulations, lengthy compound development processes and massive financial efforts (Vlieghe, Lisowski et al., 2010) all incur concern that, despite the increasing investment into research and development, medicinal innovation is declining. Especially the last decade has seen a major paradigm shift in the scope of the pharmaceutical sector, focusing more on orphan or repurposed drugs and reducing production costs, as to endure the high expenses associated with drug development. Fewer new drugs make it to the market and the patent protection of current blockbuster drugs is deteriorating, with a resulting drainage of the drug pipelines. All this may ultimately push the pharmaceutical industry towards a new frontier in modern drug development. Fresh strategies are needed to revive pharma's lost momentum and we agree with Vlieghe and coauthors (Vlieghe, Lisowski et al., 2010) that the sector's hope (partly) lies in peptides.

3. Peptide discovery

3.1. *Natural sources*

Nature harbours an impressive variety of biologically active peptides expressed in virtually all living species and, therefore, represents one of the most promising sources for peptide drug discovery (see also www.NP2D.com).

Within the multicellular body, peptides exert diverse biological roles, most prominently as signalling/regulatory molecules in a broad variety of physiological processes, including defence, immunity, stress, growth, homeostasis, and reproduction [24].

Through evolution, numerous peptides have evolved to exhibit their 'natural' bioactivity outside of the producing organism. Many of these have been isolated and characterised from the skin of frogs and toads [49,55]. These genetically encoded compounds have been shown to protect and defend their manufacturers against many foes, both predators and pathogens [13]. Hitherto, over 300 antimicrobial peptides have been identified from amphibians that hold promise for future antibiotic research and development [33].

Intriguingly, many externally active peptides have evolved as means of active predation, especially in venomous animals such as spiders, snails and snakes (see [64]). While the toxicity arises from interfering with neuronal transmission (blocking synaptic signalling, ion channel; e.g. conotoxins) or, in general, disrupting critical biochemical signalling networks within the prey's body [61], low doses of these peptides can actually counteract disturbances from diverse disorders. Accordingly, toxic peptides may aid in treating pain [41], neurological and cardiovascular diseases, diabetes and cancer [32]. A prominent example is the type 2 diabetes drug *Exenatide*, a synthetic version of a glucagon-like peptide-1 analogue found in the venom of the Gila monster *Heloderma suspectum* [7].

As bioactive peptides obtained from natural sources have been subject to aeons of selective pressure, they show considerable plusses over artificially/chemically conceived peptide-like compounds. Namely, they excel in stability and target affinity, both of which are extremely challenging to achieve or reproduce through rational peptide design, screening of libraries of randomly composed peptides or peptidomimetics. Although we appreciate the intelligence of peptide medicinal chemists, and other traditional (bio)chemistry based pharmacologists, we believe that much is still to be discovered from the natural bioactive peptides used all over the biological taxonomy (from microorganisms over plants to animals). With so many of these being used as drugs by so many different species for so many different purposes, it is clear that mankind can still learn a lot from the implied biology. We would, therefore, wholeheartedly support an adjustment of the name of the 'Natural Peptides to Drugs' NP2D discussion forum to 'NP4D' (Natural Peptides for Drugs).

3.2. *Peptidomics*

To date, one only begins to grasp the magnitude of peptides occurring in nature, a considerable share of which potentially offers therapeutic or diagnostic merit. Therefore, more emphasis at studying the seemingly endless variety of natural peptides and their bioactivities, is definitely justified. This is exactly what the recent science of 'Peptidomics' is all about.

In the past decade, we have seen great technological strides in peptide manipulation and analytical assessment. These include advances in liquid handling devices, synthetic protein synthesis, recombinant protein expression, multispectral micro-plate technologies, mass spectrometry, liquid chromatography, cell culture methodologies, sequencing technologies, imaging tools and high throughput peptide screening protocols [6]. Coupled to these technological advances, the commercial biotechnology sector is steadily growing [6], yielding increased numbers of commercially available biochemical assays and high throughput screening tools. Whereas expensive high-tech proteomic techniques have previously mainly been adopted in big pharma and biotech, we now see that the newest technologies are becoming accessible and are being developed primarily in academia and small biotech. Given the manifold of modern tools, a new generation of modern drug designers is emerging, exploiting the potential offered by the latest developments in all relevant scientific disciplines, such as biology, biochemistry, genetics, transcriptomics, proteo/peptidomics, computer science, mathematics, and many others.

A systematic approach to identify biologically and physiologically active peptides and thus their pharmacological promise is fundamental to the study of peptidomics. This relatively young discipline aims to holistically analyse the spectrum of peptides found in any chosen organism, mostly by means of mass spectrometry (MS), tightly linked to advancements in bioinformatics technology which is essential to keep track of and cope with the huge data sets generated.

Evolution towards high-throughput MS analysis in both qualitative identification and quantification was aided by the latest improvements in ionisation (mainly electrospray, ESI) and in high speed, high sensitivity and resolution analysers such as orbitrap systems. In addition, the emergence of mass spectrometry imaging (MSI) represents one of the most fascinating progresses in this field revealing the distribution of peptides in a biological sample and consequentially inferring their potential biological source and purpose [40].

3.3. *Peptide drug candidate screening*

For a conventional drug candidate screening, the so-called 'lead discovery' process, in which traditionally large sets of typically synthetic small molecular compounds of predefined structure are pharmacologically tested, it is essential to dispose of well-characterised peptide libraries to run a peptide drug discovery programme. However, also 'reverse pharmacology' using chromatographically fractionated extracts from originally impure biological sources of 'natural' peptide mixtures, is an approach which, thanks to the technological advances in peptidomics of the past years, may prove to be much more successful compared to the past.

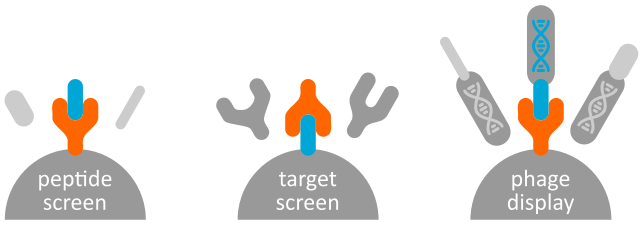

Fig. 1 – Approaches for peptide affinity analysis. Legend: cyan rods: specific target binders, light grey rods other peptides. Dark larger rods in right panel represent bacterophages. Orange forks represent specific peptide targets (e.g. a GPcR). Grey forks represent non-specific targets. Peptide affinities towards one or several targets can be investigated by immobilising either of both and identifying the binding species (e.g. via detectable tags and/or via mass spectrometry). A third option is phage display, where peptides of interest exposed on the surface of bacteriophages bind to their target. Their respective DNA sequence is contained within the phage and allows identification of the peptide.

Entirely parallel to the high throughput screening of small molecule chemical libraries, synthetic peptide chemical libraries can be produced using today's technologies. These can include natural peptide sequences, supplemented with derivatives thereof, such as naturally occurring post-translationally modified and truncated isoforms, or entirely artificial peptides. Indeed, peptides of any deliberately chosen or randomly assembled sequence can be produced by purely chemical means or by recombinant organisms. Both allow for an immense flexibility in sequence and post-translational modifications (PTMs) enabling the creation of correspondingly vast libraries which potentially contain substances of therapeutic interest. We remark here that the choice of residues is by far not limited to proteinogenic amino acids, and is basically only limited by the imagination and competence of the synthetic peptide chemist and/or peptide molecular biologist. Indeed, while chemical synthesis may integrate any compound able to bond with the nascent peptide [1], the introduction of artificially expanded genetic codes has widened the range of recombinant peptide structures by inserting non-conventional amino acids [66,52].

Three strategies for screening artificial peptide libraries can be distinguished (Fig. 1). In the first approach, peptides synthesised on a solid support are cleaved off for activity screening [26]. Secondly, peptides are assessed while still attached to the solid phase on which they were synthesised. Finally, phage display is the third approach where bacteriophages expressing the peptide and exposing it on their surface are analysed for their affinity to a selected target.

Besides the former peptide chemistry- and molecular biology-based strategies to populate peptide libraries which can be screened in straightforward pharmacology tests, also reverse pharmacological approaches starting from natural sources of biological peptide mixtures have become a very realistic alternative. Here, screenings are performed with initially uncharacterised natural peptide structures isolated and fractionated from their biological source. Biologists help to

identify the sources with the highest potential, considering the disease target aimed for. Rich mixtures of biological peptides can be found throughout the entire taxonomy, from vertebrates over invertebrates and plants to microorganisms. Classical examples are venom and defensive gland secretions which are found throughout zoology (see e.g. www.np2d.com; www.venomics.eu).

The components of peptide libraries can be assayed for their bioactivity *in vitro* (target affinity, binding kinetics, etc.) and *in vivo* (altered gene expression, cytotoxicity, etc.). Combinatorial analysis of known peptide sequences (e.g., Ala-scans) and the extent as to how changes in the primary structure affect the original biological activity, enables to reveal structure–function relationships [18]. This 'deconvolution' allows the selection of sequences for peptides with a desired activity. As such, it essentially provides the fundament for rational design of peptides with predefined biological effects.

In all cases, peptide library screenings are intended to show molecular interaction with a drug target using different visualisation technologies comprising colorimetry, fluorescence microscopy, flow cytometry and others. Classes of biomolecules which have previously been extensively investigated and successfully modulated as traditional drug targets include ion channels, nuclear or G-protein-coupled receptors, transcription factors or enzymes.

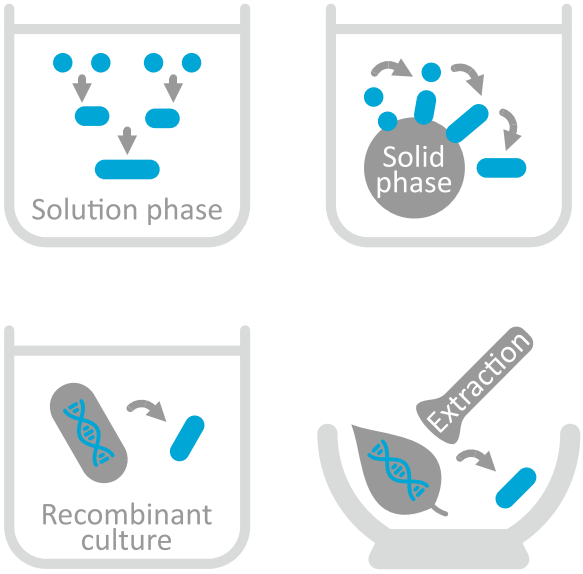

Fig. 2 – Means of producing therapeutic peptides. Peptide manufacturing can be achieved entirely through chemical synthesis, either in the solution phase (top left), coupled to a solid phase (top right) or by the combination of both. Alternatively, peptides can be produced by recombinant microorganisms (bottom left) or by extraction from their natural (plants or animal) source (bottom right).

4. Large-scale peptide production

Once a peptide has been selected for further development as pharmacon, it needs to be produced in large quantities with consistent quality, according to good manufacturing practice (GMP) rules.

The strategy for producing a peptide is largely determined by its size and chemical features. A variety of technologies such as chemical synthesis, recombinant DNA technologies, cell-free expression systems (*in vitro* translation) and transgenic plants or animals have been adopted for this purpose (Fig. 2).

Traditionally, the production of natural compounds is achieved by using a microbial or fungal strain that underwent a series of induced mutations and subsequent screenings for productivity. Through this process, the production yield may typically be raised by up to three orders of magnitude. Specifically designed strains with enhanced protein synthesis, secretion and folding capability provide a solid platform and starting point for reaching high peptide yields [34].

Large-scale operations incorporating chemical synthesis as the core production technology may be seen as an attractive alternative to existing recombinant DNA-based or biocatalyst-based methodologies [65]. Chemical synthesis can be distinguished into three major categories, namely solution phase, solid phase and hybrid approaches. Selection of the most favourable production process primarily depends on the set objective and the limitations that every method presents.

The majority of peptide pharmaceuticals are produced in high volumes using solution phase chemistry which is preferably employed for small to medium-sized peptides. Prolonged development times are a major drawback for the application of this technique especially during early clinical studies when rapid production of the desired substance is crucial. Nevertheless, significant advantages of this methodology are the well-established isolation, characterisation and purification protocols of the intermediate products [1]. On the other hand, solid phase peptide synthesis (SPPS) has enabled the production of large, complex peptides of pharmaceutical grade purity on a large scale [1,65]. Finally, hybrid processes combining the advantages of both techniques may offer even greater potential [1].

Irrespective of the upstream production method of choice, downstream processing is a vital step in the manufacturing of peptide pharmaceutical products, since it involves the critical steps associated with product isolation and purification [1].

5. Peptide drug registration

Peptides represent a special case in regulatory affairs, since, depending on its properties and manufacturing, a peptide is sometimes regarded as a conventional chemical medicinal product, and in other cases as a biological entity.

The United States Food and Drug Administration (FDA) traditionally handles peptides as conventional drugs, not as biological products [20]. This goes along with the focus of examination on the drug composition and compound structure rather than the means of manufacturing. According to the FDA, the upper size boundary of chemically synthesised peptides is at 100 amino acid residues. Exceptions, however, are made where peptides otherwise meet the statutory definition of a biological product, such as in the case of peptide vaccines.

The European Medicines Agency (EMA) does not provide such distinction based on size. Instead, peptides are simply treated as biological entities, if they are either extracted from their natural sources or recombinantly produced [15,16]. Chemically synthesised peptides, accordingly, are treated as conventional small molecular chemical entities. Nevertheless, a peptide may be regarded as significant therapeutic innovation, as for instance has been the case for Exenatide [17]. This enables the centralised approval procedure for gaining marketing authorisation in the entire EU at once.

In terms of manufacturing, only one guideline specifically addresses peptides, i.e. the "Guidance for Industry for the Submission of Chemistry, Manufacturing, and Controls Information for Synthetic Peptide Substances" from the FDA's Center for Drug Evaluation and Research [19]. It specifies that the lot release specifications should be sufficient to ensure the identity, purity, strength and/or potency of the peptide and to demonstrate lot-to-lot consistency [58].

A poignant saga illustrating the 'resistance' certain peptide compounds face on their way to the drug market, is this of magainin [42]. Even after completion of phase III, FDA in 1999 decided against approval of pexiganan (a 22mer linear peptide analogue of magainin originally identified from *Xenopus laevis*). The reason was that the trial did not show superior efficacy over other antibiotics used in the indication under investigation. The fact that magainin, because of its unique mode of action (polycationic peptide with hydrophobic residues forming transmembrane pores in the highly negatively charged bacterial cell membranes, bleeding the microbial cell to death), is virtually impossible for a bacterium to develop resistance against, was not taken into account. Whereas typically, regulatory issues tend to elongate the duration of clinical trials by a significant amount of time, the FDA launched the Antibacterial Drug Development Task Force in September 2012 [21] in order to increase the efficiency of antibiotic development including the clinical trial design. Similar developments are on-going in Europe by the EMA; new guidelines are released which concern the clinical criteria for evaluating antimicrobials [22,31].

The currently changed attitute towards antimicrobial peptides is illustrated by surotomycin (developed by Cubist Pharmaceuticals). The compound, a lipopeptide very comparable to vancomycin (a currently available common antibiotic; [35]), is now in phase III against *C. difficile* infections and has been designated '*qualified infectious disease product*' (QIDP) status under the FDA '*generating antibiotic incentives now*' (GAIN) act. This means that priority review, fast-track status, and a five year exclusivity after license are applicable [22].

6. Peptide pharmacodynamics and pharmacokinetics

Many of the challenges peptides face in the drug development process occur in the preclinical development phases. Preclinical biological activity is evaluated using *in vitro* and *in vivo* pharmacology assays that determine the effects of a product (pharmacodynamics) related to its clinical activity. Additional important pharmacological parameters include the pharmacokinetics (PK, absorption, distribution, metabolism and excretion; ADME) [38]. Determination of all these parameters to the full extent is especially challenging in the case of synthetic or recombinant peptides (or proteins), as they usually show patterns deviating from more traditional small molecule pharmaceuticals [60] which typically reach their (often intra-) cellular targets by diffusion into all cells of the body. This is quite different from the regular bioactive peptide which exerts its effect through binding with a cell surface receptor, after having successfully overcome the challenges inherent to reaching the general circulation (see also below "Peptide drug formulation"). Here peptides have a slight disadvantage compared to conventional small molecule drugs, which not seldom are selected for their easy crossing of cellular membranes/barriers.

Pharmacology studies of peptide drug candidates are still very tough, as the targeted analytical identification and quantification of peptide drug substances from complex matrices is still strongly limited [12]. The actual analytic screening for peptides in preclinical studies is mainly based on detection via immunological assays. Although these methodologies do offer a high throughput, they suffer from major limitations in terms of specificity and dynamic range [25]. At the same time, the development of new protocols and assays is currently still extensive and laborious [12]. Consequently, in terms of reliability and economics, analytical techniques suitable for routine targeted peptide metabolism ('bioanalysis') studies still need to be developed.

A solution to cross this technological chasm may come from the most recent advancements in peptide mass spectrometry (MS), expanding the tool box of MS technologies. The optimal integration of innovative instrumentations, protocols and efficient data treatment tools available to date will lead to dedicated workflows to analyse specific peptides in biological complex matrices [12].

Aspects like internal (isotopically labelled) standard availability for the generation of reliable calibration curves, together with instrumental setup are capital in peptide quantification and ADME studies. Moreover, the panorama of data acquisition is complicated by the diversity of mass spectrum deconvolution technologies and data mining software. Yet, several examples exist of how the most recent MS evolutions can be successfully applied in the context of peptide identification and quantification from complex biological matrices. Technologies like selected reaction monitoring (SRM) and high resolution mass spectrometry (HRMS) actually emerge as very promising [12], especially in the investigation of peptide metabolism. These advancements, hand-in-hand with adequate sample preparation workflows, including high and ultra performance liquid chromatography – biofluids such as blood, cerebrospinal fluid or others comprise very distinct analytic environments – may help solve this still major bottleneck in pharmaceutical peptide metabolism studies.

Finally also the above mentioned mass spec imaging (MSI) technology is very promising as tool to pre-clinically map the distribution of drugs and their metabolites in the body, replacing the current autoradiography (MSI does not require a radiolabel). Whereas MSI is already being successfully used in small molecular drug PK (see e.g. [48]), it still needs to be successfully demonstrated for peptide drugs and their metabolites.

7. Peptide drug formulation

As promising as peptide drug candidates may be, a number of conceptual limitations remain associated with the traditional image of a peptide as a typical medicine. This perception is largely substantiated by Lipinski's "rule of five" which summarises the ideal pharmacokinetic properties of the most successful (chemical) drug candidates of the original pharma industry. This decree dogmatises that peptides are less likely to pass through the gastrointestinal (GI) tract wall as compared to small molecules due to their larger size and comparatively low solubility [65].

Many challenges peptides experience on their way to becoming an effective drug originate in their physicochemical properties which together result in a poor oral bioavailability. Due to their hydrophilicity, peptides exhibit limited ability to cross physiological barriers. In addition, peptides are confronted with efficient hepatic and renal clearance. Even once inside the systemic circulation, peptides typically have rather short half-lifes due to aggressive degradation by a multitude of proteases [3]. These aspects, intrinsic to the chemical nature of peptides, have compelled cumbersome administration routes such as direct injection of repeated doses, which in turn resulted in low patient compliance [3].

Aside the classic subcutaneous, intramuscular and intravenous administration, alternative routes have been developed, including the mucosal track (nasal spray, pulmonary delivery or sublingual delivery), the oral route (GI tract penetration enhancers, protease inhibitors or carriers) and the transdermal path (patches; [3,65]). For instance, a peptide pill for oral administration is developed (Enteris Biopharma) the coating of which effectively protects the active substance from digestion in the stomach, allowing its release in the duodenum. A number of excipients protect the peptide against peptidases and facilitate its paracellular uptake through the intestinal wall into the systemic circulation [57].

Benefits of the pulmonary intake are a very large available absorptive and highly permeable surface, extensive vascularisation, with concomitant rapid onset of pharmacological action, a more uniform distribution of the drug product and a more sustained drug release which allows a reduction of the dosing frequency [2,28]. Like other parenteral administrations, the first pass metabolism is avoided. Also a 10–200 times higher bioavailability (higher drug product plasma concentrations) can be achieved because of the smaller volumes used [56,63,2].

Besides alternative delivery systems, various formulations have been devised in the past, trying to deal with the aforementioned physicochemical limitations, to help advance promising peptide drug leads into pharmaceutical development. Again, most of these medicinal preparations are chemistry- and nanophysics-based. Indeed, chemical incorporation of sugars like trehalose, sucrose, maltose, glucose, of salts like potassium phosphate, sodium citrate, ammonium sulphate and/or of other agents such as heparin into the prospective formulations have been found to increase the solubility and *in vivo* stability of peptides. Employment of cationic and anionic surfactants such as cetrimide and sodium dodecyl sulfate (SDS) have shown enhanced transportation

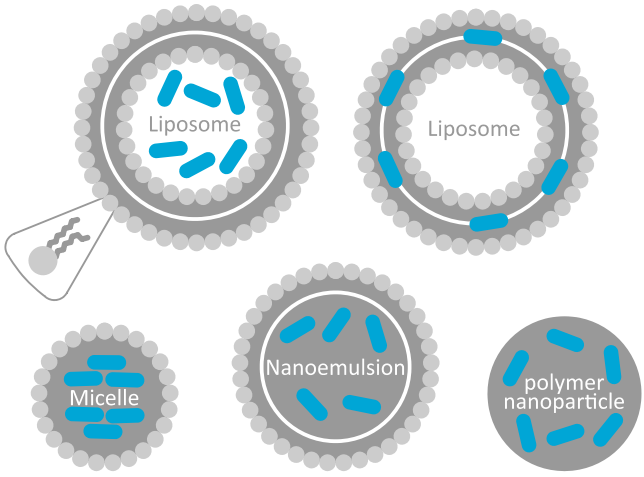

Fig. 3 – Modern formulations for the protected and optionally targeted delivery of therapeutic peptides. Several modern formulations offer enhanced tissue penetration, delayed decay of therapeutic peptides and targeted delivery by exposing specific receptor ligands. Liposomes (top) harbour lipophilic peptides within the lipid bilayer, while hydrophilic peptides are stored in the aqueous core. On the other hand, micelles (bottom left), nanoemulsions (bottom centre) and polymer nanoparticles (bottom right) incorporate lipophilic peptides into their core. The insert at the top left liposome represents a typical cell membrane amphiphilic (phospho)lipid with light grey denoting the polar head group and dark grey indicating the apolar moieties.

of peptides across bodily membranes. Considerable increase in resistance against proteolysis is often achieved through co-administration of protease inhibitors such as sodium glycocholate, camostat mesilate or bacitracin. Moreover, the attachment of polymeric molecules such as polyethylene glycol (PEG) and polyvinylpyrrolidone (PVP) and even the encapsulation of the peptide into nanocarriers are employed for extended bioavailability (Antosova, Mackova et al., 2009).

Nanocarrier technology indeed seems a promising innovation to increase peptide pharmacodistribution through, for example, nanoparticles, liposomes and micelles (Fig. 3). These are thought to effectively create a closed carrier that protects the active compound from destabilising external threats such a peptidases. In combination with pulmonary inhalation, nanocarriers have the benefit of prolonged drug release due to the combination of peptide protection by the carrier's shell with carrier accumulation [2].

In general, an ideal nanocarrier should be composed of inert and biodegradable material and be able to efficiently encapsulate and protect the peptide against degradation while at the same time maintaining proper drug product targeting [28]. Compositions of polymeric formulations can be used to tune the biological behaviour of nanocarriers by modulating compound properties such as mucoadhesiveness [8]. An example is the cationic polysaccharide chitosan, the ionic interactions of which with the negatively charged sialic acid groups in mucin provide exceptional binding. In combination

with its ability to open tight junctions, chitosan effectively favours the utilisation of the paracellular pathway [11,53,2]. Hybrids can be created by combining chitosan with other polysaccharides or oligosaccharides in an attempt to further improve the physical properties and pharmacological performance of peptide drug formulations [23,28].

Other synthetic polymers such as polylactic acid (PLA) and poly(lactic-co-glycolic) acid (PLGA) have been found viable substitutes. Their consistency in terms of peptide drug product release in combination with good biodegradability guarantees optimal safety. Accordingly, the FDA has already approved a number of respective marketed products and clinical applications [11,2,28].

Liposomal nanocarriers are composed of one or multiple phospholipid bilayers. Specific ligands conjugated with modified PEG molecules in the shell offer targeted delivery [30,28]. However, liposomes often face varying instabilities in biological fluids [2]. Nanoparticles which consist of a solid (both at ambient and body temperature) lipid matrix dispersed in an aqueous phase may offer an alternative. One distinguishes solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs). While SLCs are rigidly structured, NLCs consist of both solid and liquid lipids allowing for an increased loading capacity [39,43,2]. Finally, self-nanoemulsifying systems, i.e. isotropic mixtures of oil and surfactant forming thermodynamically stable oil-in-water emulsions, may have potential for enhanced oral bioavailability of poorly water soluble peptides [28].

Besides these modern (nano)technology-inspired formulations, we here would like to draw the attention to the wide variety of bioactive peptide delivery systems found in nature. Evolution has designed direct injection devices, very much like injection needles, such as fangs or venom teeth in various reptiles, harpoon-like radulas in snails, or claws. However also organisms lacking these appear to successfully use bioactive peptides in their gland secretions. A careful analysis of the composition of peptide-containing venom and defense glands in animals like amphibians, may, therefore, give hints as to how potential therapeutic peptides can be successfully formulated. The exosomal release which is observed from various venom glands in a way is comparable to the micellar encapsulation of peptides described above. Biology also hints to additional tricks to ensure effective use of peptide bioactives. In nature peptides are almost never secreted on their own, but always in cocktails, basically a mixture of three types of peptides. These not seldom contain polycationic amphipatic (poly)peptides (sometimes catalogued as 'antimicrobial peptides' (AMPs)) which could serve as membrane penetrating delivery systems. Co-secretion of 'peptidase inhibitors' is also a strategy seen in various animal defense glands. Finally various (often post-translational) modifications on the true 'bioactive peptides' fit in a strategy to render the (active part of the molecule) improved/prolonged stability as well as tighter receptor binding. These include sequence elongation protecting the bioactive site from rapid exoprotease digestion, C-terminal amidation and/or N-terminal ring formation (pyroglutamic acid from glutamine) for charge suppression (reduction of hydrophilicity), disulfide bridge formation for increased structure preservation and rigidity, and isomerisation of selected peptide terminal residues (from L- into D-amino acid) for enhanced exopeptidase resistance. Examples can be found in the defensive secretions of *Phyllomedusa burmeisteri* and *Bombina variegata*, as described elsewhere in this special issue [47].

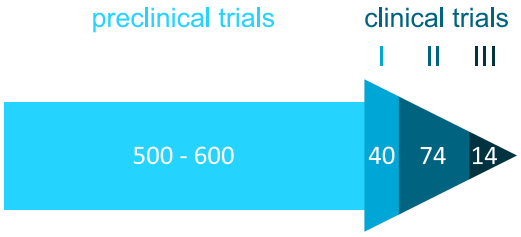

Fig. 4 – Graphical representation of number of peptides in preclinical and clinical development as of 2013 [31].

8. Clinical development

The therapeutic peptide development is growing. In 2011 alone, there were between 500 and 600 peptides in pre-clinical phases (Fig. 4; [31]). The year 2012 has proven to be another milestone for the peptide pharmaceutical sector, with 5 and 6 peptides meeting market approval respectively in Europe and in the USA. This was the highest number of approvals ever achieved for new biological entities (NBEs) in one year [29] which renders some optimism to the sector. This optimism is confirmed by the statistics, as the regulatory approval rate for peptides is around 20%, versus 10% for small molecules [31]. In addition, the number of peptides per year entering clinical trials has steadily increased from 1 in 1970 to currently around 20 [31].

As of April 2012, the clinical pipeline for peptide drugs was composed of 128 peptide candidates. These included 40 in phase I, 74 in phase II and 14 in phase III (Fig. 4). The robustness of this pipeline is largely due to the notable expansion in the field of peptide therapeutics during the late 1990s and 2000s, ultimately leading to the number of approvals observed in 2012. Considering the general failure rate of the clinical pipeline [29], these numbers prove very promising.

In Phase I, the most represented indications are pain (more than 30%), cancer and cardiovascular diseases. In Phase II and III, in which cancer is the leading target (accounting for more than 15% and 40%, respectively), one finds, next to pain and infectious diseases, also indications not much represented in the current market, such as dermatology, allergies, and CNS disorders [29].

8.1. *Typical (GPC) receptor binding peptides*

One area of research that has shown promising development are the use of peptide therapeutics in treating type 2 diabetes (targeting the glucagon-like peptide 1 receptor). Already three peptides have received approval in 2012, with 14 working their way through the pipeline. A most exciting aspect of these peptide drug candidates is the variety in drug formulation of molecular formats (with peptides being covalently linked to small molecules, carbohydrates, lipids, biopolymers, polyethylene glycol or proteins (see above)) and

their mechanisms of actions (including specific cell-targeting peptides and cell-penetrating peptides) currently being elucidated. Thus, substantial efforts are being made to modify molecular properties of peptide drug leads to improve their functionality. For example, half-life extension was the rationale for four peptides (CBX129801, CVX060, LAPSExd4, PB1023) in phase II, whereby peptide conjugation to polyethylene or IgG substantially increased peptide stability in circulation from minutes to days or even weeks. Improved biological barrier crossing/cell-penetration was the rationale behind the design of three other peptides (CBP501, AM111, ACT1) also in phase II. Typical amphipathic and cationic features of these peptides are enhanced by the molecular addition of cell-penetration promoting sequences such as the transcription transactivation (TAT) sequence from the HIV virus [36].

8.2. *Antimicrobial peptides*

With the frightening advent of global increase of microbial resistance to conventional antibiotics, the search for alternatives has become of utmost importance, and the industry as well as the regulatory authorities are realising the potential of antimicrobial peptides (see also above).

A recent overview of antimicrobial peptides currently in clinical phases by [22], includes 10 compounds (developed in 10 different companies both in North America and in Europe). In this list we see Pexiganan (magainin, see above) reappear, albeit no longer developed by the original company Magainin Pharmaceuticals, but by Genaera, the name the enterprise was re-baptised in.

8.3. *Peptides as vaccines*

An entirely different sort of therapeutic peptides are the peptide vaccines. These peptides, representing inactive, non-virulent fragments of pathogen proteins are becoming gradually more mainstream. On-going trials are spanning all phases of clinical development. The list of benefits for especially synthetic peptides as vaccines includes their ease of quality control, chemical stability and the absence of oncogenic, toxic or infectious material [51]. Whereas not many successes have recently been achieved by employing peptide vaccines [44], the advent of personalised peptide vaccination (PPV) could herald changing times. Taking factors into account such as the human leucocyte antigen (HLA) system and pre-existing host immunity [44], PPV may have a future, providing current phase III trials are as successful as they promise to be [44,67].

8.4. *Future perspective*

Some very promising peptides to watch out for in the coming years are now in late phase clinical trials. About half of them are intended for oncology, metabolic or cardiovascular treatment as well as for remedying infectious diseases [31]. Especially for illnesses requiring prolonged therapy, peptides have a competitive advantage over conventional small molecule drugs. In terms of general safety, peptides have a comparatively small toxicological footprint. Due to their extremely high specificity for their intended target (the peptide receptor), in combination with the fact that they are extracellularly active (not requiring systemic diffusion and hence extreme dilution over all cells), much lower amounts can be formulated. Moreover after peptide receptor binding and signal triggering, highly efficient peptide catabolism through proteolytic degradation yields simple amino acids, which are recycled in the body in everyday metabolism such as protein synthesis. Compared with other small molecular chemical entities, which often represent extreme challenges to the body's detoxification mechanisms, peptides suffer from little if any accumulation in the body, nor in the environment. In contrast to various poorly metabolising or absorbing small chemical drugs, no surface water pollution occurs by residual active substance excretion into the environment after peptide drug use.

It can be concluded that the pharmaceutical peptide pipeline is strong and stable, with several candidates approaching drug approval status. The commercial value of the therapeutic peptide market is well established (see below). However, the recent and nascent approvals promise to substantially increase the market value of peptide therapeutics in the coming years, in the areas of diabetes, oncology [29,31] and beyond.

9. Peptide patents

Several sources report that the number of patent applications involving peptide-related technology has significantly grown in the last decades. A recent update on patent applications is available [46].

An illustrative example of a patent application involving peptides with pharmaceutical potential is provided by the approved patent EP1590458B1 "Bradykinin B2 Receptor Antagonist Peptide from Amphibian Skin" [54]. The patent discloses the sequence of a peptide (kinestatin) isolated from toad (*Bombina maxima*) defensive skin secretion, while claiming the protection for kinestatin analogues, prodrugs including the peptides, fusion peptides and multimeric peptides. At the same time, it gives a broad indication of the potential therapeutic application areas, including cardiovascular disorders, inflammation, asthma, allergic rhinitis, pain, angiogenesis and the like, glaucoma, hydrocephalus, spinal cord trauma, spinal cord oedema, neurodegenerative diseases, including Alzheimer's disease. The claims comprise the application of the patented molecules wherein said peptides are present in or conjugated onto a liposome or microparticle that is of a suitable size for intravenous administration, but that lodges in capillary beds, thus opening the road to overcoming potential administration hurdles. This patent application provides the reader with a general framework for patent application involving peptides, namely disclosure of the main amino acid sequence motif, examples of analogues, prodrug derivatives and a broad definition of therapeutic areas.

Within the Cooperative Patent Classification (European Patent Office, 2013), category A61K38 includes "Medicinal preparations contain peptides" as a subdivision of category "Preparations for medical, dental, or toilet purposes" A61K. This is not to be confused with category C07K "Peptides" that is including application of peptides within several fields, such

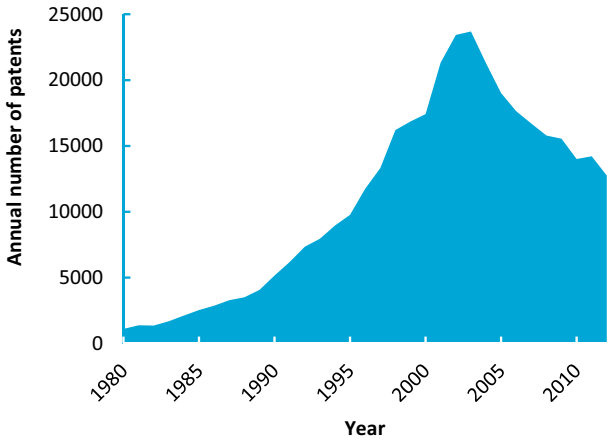

Fig. 5 – Trend in patent applications for therapeutic peptides from 1980 until 2012 [14].

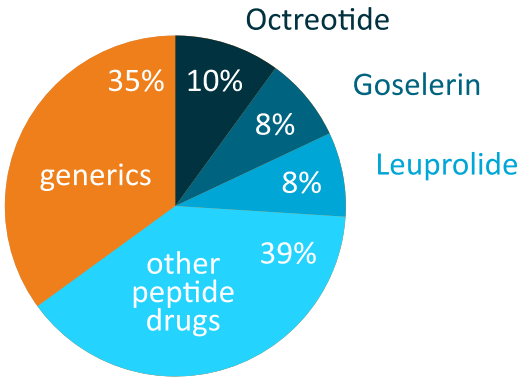

Fig. 6 – Worldwide peptide drug market distribution in 2011. Global peptides net sales amount to USD 14.7 billion (the relatively minor contribution of Octreotide to the generics market (USD 0.15 billion) was not considered).

as food. An Espacenet database query for patent applications belonging to category A61K38 for each year between 1980 and 2013 (the data for the partial year 2013 were extrapolated to the whole year) yields an interesting graph (Fig. 5; [14]).

Approximately 389,320 patent applications within the peptide fields have been published in the interval 1980–2013. Starting from the year 1996 the number of patent applications per year have invariably surmounted 10,000, a very high number, reflecting a very dynamic development of the peptide market. At the same time, it also shows an apparent peak in the number of applications per year reached in 2003 (with 23,690 new peptide-related patent applications).

Considering that an average approval time for new drugs is 10 years and that the peak in peptide patent applications occurred in the interval 2000–2005, one may predict a peak in the number of peptides entering the market as new drugs between 2010 and 2015. Apparently, an unprecedented number of peptides have in fact received market approval in the years 2010–2013 (see above). Considering that a typical protection time for a granted patent is 20 years, and that an off-patent drug tends to lose a significant part of its sales revenue to price-based competition by generics in the few years after the end of patent protection, we anticipate that peptide-based drugs may deliver rising sales revenues for pharma in a significant numbers of years to come, reasonably beyond 2020.

10. The peptide pharma market

To date, around 100 therapeutic peptides (mostly innovative synthetic ones) are on the market in the USA, Europe and Japan, including those for diagnostics applications [29]. The increasing numbers of recently approved peptides is a result of the well-filled pipeline, as described above. The market appears dominated by the three peptides Goserelin/Zoladex and Leuprolide (two gonadotropin releasing hormone agonists, used in hormone-sensitive breast and prostate cancer), and Octreotide (a somatostatin mimic used against various tumours), which account for annual sales (2011) between USD 1.2 and 1.4 billion [45,4,59], in total around 25% of the global peptide market [62].

A clear trend over the past years is the continually expanding contribution of peptides to the worldwide pharmaceutical market. Of a global market worth USD 956 billion in 2011 [27], the peptides' share was about USD 14.4 billion [29,62], comprising around 1.5%. The major contributor is the US market, which accounts for 40% of the global peptide sales. In 2011, the cancer sub-market was the largest, representing 21% of the total peptide market, followed by metabolic disorders, gastrointestinal diseases and respiratory indications [62] with 86% of the approved peptide drugs working through the parenteral route. As indicated above, however, alternative routes are foreseen to expand [29]. The total market potential of peptides may be significantly larger, since, in addition to the therapeutic pharmaceutical market, peptides are expected to contribute more and more in other markets such as the nutraceutical business [5].

In the current stagnating pharmaceutical industry, peptides are considered to have added value, by representing a potential solution to more efficacious disease treatment. Already today they appear mature compounds addressing unmet medical needs, and accelerating the personalised medicine model [10]. In addition, peptides promise to combine the lower production costs of conventional (small molecular chemical) drugs with the high specificity of (the larger) biological entities.

Importantly, the proportion of peptides in pharma is anticipated to increase, since it is estimated to grow faster (9.4% annual growth in 2012–2018) [62] than the global industry (3–6% annual growth in 2012–2016) [27]. Not only is the number of approved peptide drugs expected to grow but also the diversity of treated indications.

It is remarkable that generic sales already account for a considerable portion of the peptides' market. In 2011, generics represented 35% of the peptide market, with three of the five top selling peptides being generics [5,62]. Octreotide for instance has a generic version accounting for USD 0.15 billion annual sales [9]. In comparison, generic sales in the global pharmaceutical market account for around 21% and biosimilars in the biologics sub-market for around 0.4% in the same year [27].

Summarising, the peptide market today, although still depending on a few blockbusters and mature drugs as obvious from the contribution of generics to the global sales (Fig. 6), is predictably increasing. The imminent pipelines indicate a bright future for peptide pharmaca, with numerous innovative peptides on the verge of approval. This endorses peptides as firm candidates to contribute to the growth and innovation of the future pharmaceutical industry.

11. Conclusion

Therapeutic peptides have spent decades as niche products, while the pharmaceutical industry focussed on small molecules as medicinal agents. Given the increasing challenges with the latter compounds, drug developers are turning back to the small amino acid chains. Whereas peptides have been deemed unsuitable for a long time, modern formulations and peptide drug desings have achieved to circumvent their weaknesses to clearly reveal more than a few advantages of these molecules. For example, the original need for injecting peptides like insulin is fading, with progressively more patient-friendly administrations being developed. Additionally, today's society which critically judges the undesirable side effects as well as environmental impact of candidate medicines should embrace the safety provided by peptides.

Whereas, therapeutic peptides originally were developed to replace their endogenous lack, the spectrum of available candidate peptide drugs is by far not limited to the human peptide pool. Indeed, through the modern tools of peptidomics, bioactive peptides from multifarious organisms are being discovered. Nature certainly still harbours a virtually infinite array of potential peptidic medications that await (human) pharmacological characterisation. At the same time, the methods of peptide synthesis have evolved to permit highly efficient production of remarkably long and heavily modified compounds.

In the light of these advances, the recent rise of peptide drugs is not a surprise at all. At first glance, the decline in therapeutic peptide patent applications after a prior peak about 10 years ago may seem discouraging, but this does not necessarily mean that the market is reflecting this trend. On the contrary, a large number of clinical trials of peptide drug candidates is conducted to date and the market is growing steadily. Given these premises, we anticipate a bright future for therapeutic (as well as diagnostic) peptides.

Transparency document

The Transparency document associated with this article can be found in the online version.

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